nucleosomes and their modifications function as epigenetic information. It will be important going forward to extend this type of analysis to larger eukaryotic genomes, where the influence of transcription on chromatin structure is less pervasive. It will also be important to develop systems to map histones from a single nucleosome through replication at high resolution, and to determine if dispersal is actively regulated to be more restricted at some locations in the genome than others.

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DOI: 10.1016/j.cub.2011.06.062

Comparative Anatomy: All Vertebrates Do Have Vertebrae

In contrast to lampreys and jawed vertebrates, hagfishes were thought to lack vertebrae. Now, long overlooked vertebral rudiments have been analysed in hagfish, suggesting that vertebrae existed in the last common ancestor of all vertebrates.

Philippe Janvier

Living vertebrates fall into two major groups, jawless and jawed vertebrates. When Linnaeus [1] defined the zoological group we now call ‘vertebrates’, he referred to them as ‘*vertebrata craniata*’, that is, animals with a vertebral column and a skull. At that time, he considered that lampreys, although lacking jaws, could be some kind of ‘degenerate’ cartilaginous fish, possibly allied to sharks. However, he was hesitant about the systematic position of hagfishes, and first considered them as ‘intestinal worms’, because hagfishes are scavengers and are often found inside dead fish. Later, Abildgaard [2] showed that hagfishes are actually fishes and somewhat similar to lampreys. Soon after, Dumeril [3] confirmed this anatomical resemblance, and therefore classified lampreys and hagfishes in the same group, called Cyclostomi (cyclostomes), because they both lack paired fins and true jaws, and share a single median nostril, a tongue-like feeding device armed with horny teeth, pouch-shaped gills and an entirely cartilaginous skeleton.

With the rise of evolutionary thought, the cyclostomes were then regarded as an early offshoot of the vertebrate tree, which might have diverged before the jawed vertebrates, or gnathostomes. However, Linnaeus’ old intuition that lampreys were somehow ‘degenerate’ fishes was still latent in the mind of the zoologists of the nineteenth century, who generally thought that hagfishes were even more ‘degenerate’ than lampreys. This is also how they interpreted the apparent lack of any vertebral skeletal elements in hagfishes; a question that has been revisited with surprising results in a recent paper by Ota *et al.* [4]. On the basis of developmental and gene expression data, the authors conclude that hagfishes do indeed possess what looks like rudiments of vertebrae. These rudiments form from embryonic tissues that express cognates of *Pax 1/9* and *Twist* genes, exactly like those which give rise to the vertebrae in jawed vertebrates.

Paraphyletic Cyclostomes?

In the early twentieth century, some cyclostome-like features, such as a median nostril, were discovered in the 425–360 million year-old ostracoderms, an ensemble of fossil, armoured, jawless and essentially marine vertebrates. This discovery seemed to support the view that hagfishes and lampreys were derived — perhaps independently — from these Palaeozoic fishes, through an extensive loss of the dermal skeleton, a simplification of the braincase and a loss of paired fins [5]. Yet, all jawless fishes, fossil and recent, were regarded as belonging to the same clade (monophyletic group), the Agnatha, a sister group to the gnathostomes.

This became the predominant view during most of the twentieth century, until the 1980s when the morphological distinction between the jawless and jawed vertebrates began to progressively break down. In palaeontological circles, a first surprise came with the discovery of the first fossil lamprey, *Mayomyzon*, from 300 million year old sediments from the USA [6]. This age makes them merely 70 million years younger than the last ostracoderms from which cyclostomes were supposed to be derived. The striking resemblance between

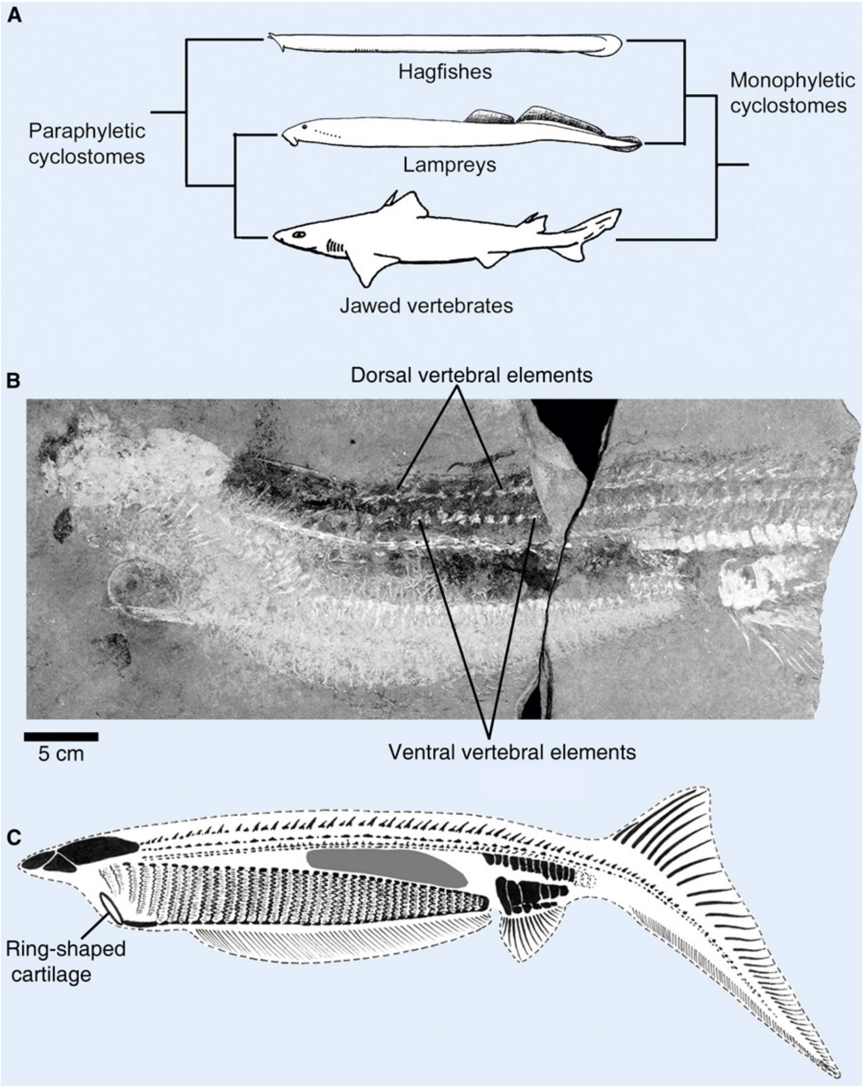

Figure 1. Cyclostomes and vertebrate evolution.

(A) The phylogenetic relationship between living vertebrates is now well corroborated by phenotypic and molecular data, but whether cyclostomes (hagfishes and lampreys) form a paraphyletic (left) or monophyletic (right) group has been a matter of debate. Cyclostome monophyly is now strongly supported by molecular evidence. However, very little is known of the cyclostome fossil record, and fossil lampreys or hagfishes are poorly informative. (B,C) Among the possible early stem cyclostomes, *Euphanerops longaevus*, a 380 million year-old jawless fish from the Devonian of Canada, is preserved in exceptional environmental conditions. Although producing no bone, the skeleton of some large individuals sometimes preserves calcified cartilage (B), and allows a reconstruction of the entire skeleton (C). This fossil jawless vertebrate displays a huge branchial basket and a ring-shaped cartilage that recalls the annular cartilage of lampreys (C), but it also possesses dorsal and ventral vertebral elements (B) that are strikingly similar to those that are presumed by Ota *et al.* [4] to have been present in the last common ancestor of all vertebrates. Adapted with permission from [17].

Mayomyzon and a modern lamprey raised doubts about the presumed 'degeneracy' of lampreys, while the rise of the cladistic method of phenotypic character analysis increasingly supported the hypothesis that ostracoderms might in fact be stem gnathostomes; that is, they are extinct members of a grade (a paraphyletic group) that are more closely related to gnathostomes than to either hagfishes or lampreys. In other words, the cyclostomes probably never developed a bony skeleton, whereas ostracoderms are jawless stem gnathostomes that diverged after the rise of bone and dentine [7–9].

Around the same time, anatomists and physiologists began to reconsider the characters of hagfishes, lampreys and gnathostomes in the light of the principles of phylogenetic systematics. In particular, many of the odd anatomical and physiological characters of hagfishes, long regarded as evidence for their 'degeneracy', became progressively regarded as possibly primitive conditions relative to their homologues in lampreys and gnathostomes [8–10]. Although considering the complex cyclostome tongue-like feeding device as a shared primitive vertebrate character seemed rather counter-intuitive [11].

One of the most striking of these presumed degenerate features was the lack of any vertebral elements in hagfishes, whereas lampreys clearly possess a series of cartilaginous elements that flank the notochord and spinal cord dorsally, and are regarded as homologous to the similarly-positioned elements (basidorsals and interdorsals) of gnathostomes, which give rise to the vertebral neural arches. Again, character analyses generally showed the derived phenotypic characters shared by lampreys and gnathostomes outnumbered those shared by lampreys and hagfishes, thus supporting the theory that lampreys were more closely related to the jawed vertebrates than to hagfishes. Therefore, it was suggested that the name 'Vertebrata' should only refer to the clade including lampreys and gnathostomes, which both possess vertebral elements and whose sister group are hagfishes, all three taxa being included in the Craniata [7].

This hypothesis of cyclostomes as a paraphyletic (Figure 1A) — rather than a monophyletic — group was indeed quite exciting because it entailed that many of the anatomical characters shared by hagfishes and lampreys, such as the median nostril, pouch-shaped gills and complex tongue-like feeding device, were shared ancestral (plesiomorphic) characters of vertebrates. Thus, they could have been present in the last common ancestor of all vertebrates, enabling a theoretical 'reconstruction' or re-imagination of what this ancestor might have looked like.

Back to Cyclostome Monophyly — and 'Degeneracy'?

The enthusiasm for a research program on living cyclostomes and the reconstruction of the vertebrate morphotype soon cooled down in the late 1990s, in particular through the progress in molecular sequence-based phylogenetics. Despite some methodological reservations, these molecular approaches provided increasingly strong support for cyclostome monophyly [12,13] (Figure 1A). Perhaps the most convincing evidence for this came from the analysis of microRNAs (miRNAs) [14], which provided evidence for strong cyclostome phylogenetic 'signatures'. In addition, miRNA expression in various organs of gnathostomes and lampreys indicated that the latter may have lost many phenotypic traits since the two groups diverged, between 360 million years ago at the latest, and probably much earlier, about 500 million years ago.

Although expression of these miRNAs could not yet be studied in hagfish embryos [15], these new data strongly support the hypothesis that cyclostome ancestors were probably more complex than the living forms [14]. Recently, Ota *et al.* [4] have revisited the question of the alleged lack of vertebral elements in the hagfish *Eptatretus burgeri*. They investigated a series of minute cartilaginous nodules that line the notochord ventrally in the caudal region of the embryo and had been mentioned long ago but overlooked since [16]. These nodules form from mesenchyme of the ventromedial part of the somites, which express *Pax 1/9* and *Twist* genes. Homologues of these genes are expressed in the corresponding part of the sclerotomes in jawed vertebrates, from which the ventral elements of the vertebral column arise — that is, the basiventrals and interventrals that persist in the adults of most early gnathostomes. This is strong evidence for the homology of these elements between hagfish and gnathostomes Yet, unlike lampreys and gnathostomes, hagfishes show no sign of corresponding dorsal elements. Therefore, Ota *et al.* [4] suggest that both the dorsal and ventral series of vertebral elements were initially present in the common ancestor of all vertebrates, with the dorsal one having been lost in hagfishes, and the ventral one in lampreys.

Among fossil jawless vertebrates, only the 380 million year-old *Euphanerops* (Figure 1B,C) seems to display dorsal and ventral series of vertebral elements. Its dorsal elements strikingly resemble the basidorsals and interdorsals of lampreys, with the ventral ones extending ventrally to partly surround the dorsal aorta, as also observed in the hagfish embryo [4,17]. Contrary to 'ostracoderms', *Euphanerops* produced no bone, and all the skeletal elements observed in large individuals are still preserved because they consist of calcified cartilage (Figure 1B). Its head also displays a peculiar ring-shaped cartilage, which is suggestive of the annular cartilage that arms the oral disc of lampreys (Figure 1C). This cartilage is practically the only character of *Euphanerops* which suggests a relationship with lampreys, except for a vaguely similar overall body shape. However, the structure of its axial skeleton is strikingly similar to that suggested by Ota *et al.* [4] for the last common ancestor of vertebrates.

The phylogenetic relationships of *Euphanerops* are still obscure, but it is tempting to consider it as either an early lamprey relative or a stem cyclostome. Whether cyclostomes are monophyletic or paraphyletic, their stem, or that of either hagfishes or lampreys, is still out of reach for palaeontologists. Thus, any developmental data, such as those provided by Ota *et al.* [4], will be invaluable for comparative anatomists whose goal is to elucidate vertebrate phylogeny and reconstruct the vertebrate ancestor.

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DOI: 10.1016/j.cub.2011.07.014

Subcellular Positioning: Unstable Filaments On the Move

A key question in cell biology is how proteins and entire protein complexes localize to defined subcellular positions in non-compartmentalized cells or within cell compartments. A recent report involving computational modeling and live-cell imaging suggests that dynamically unstable protein filaments provide an adaptable and versatile positioning system.

Peter L. Graumann

The number of examples of proteins and organelles that are positioned in the cell centre in bacterial or eukaryotic cells is growing — e.g. the nucleus in fission yeast [1,2], the replication machinery in *Bacillus subtilis* [3] and